and tubulin surface, two of which are involved in stabilizing the extra turns of switch II helix (α4) formed toward the nucleotide-binding pocket. In contrast, only few salt bridge formations are possible in ADP state, explaining why ADP release causes specific and tight binding to microtubule. The structural change of α4 promotes hydrogen bond and hydrophobic interactions of highly conserved residues in α4 with switch II loop, pulling switch II loop away and promoting ADP release from nucleotide pocket. ADP release and ATP binding cause rotational movements of α4 and also rotational movements of nucleotide-binding P-loop and its surrounding elements. These nucleotide-dependent domain motions alter the mobility of the neck linker, providing structural basis for how kinesin's two motor domains coordinate to move processively.

1923-Pos

Probing the Mechanism of Kinesin-1 Motion in Three Dimensions Using the Photonic Force Microscope

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Kinesin-1 is a molecular motor essential for cellular function. It transports components around the cell by a processive movement along microtubules while hydrolysing ATP. Although extensively studied by a variety of techniques, the mechanism used by these single-molecule motors to produce this efficient motion on the nanometer scale is not fully understood.

In our investigations we use the Photonic Force Microscope (PFM) to trap and track a 500nm bead attached to a kinesin motor as it interacts with a microtubule *in vitro.* The PFM is an optical trap capable of recording a trapped dielectric particle's motion in three dimensions with nanometre spatial and microsecond temporal resolutions. Using the data recorded we can infer information about the molecular motor's position and its mechanical properties. By characterising different conformational states of the kinesin molecule from its changing mechanical properties as it processes, we expect to learn more about the cycle of events that make kinesin movement possible.

An understanding of how nature achieves this motion on the nanoscale will help combat diseases related to kinesin's malfunction and will allow production of similar artificial nanomachines in the future.

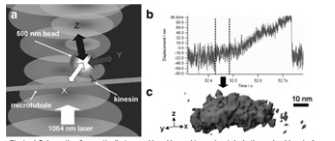

Fig.1: a) Schematic of an optically trapped bead bound to a microtubule through a kinesin-1 molecule. b) Position trace of a bead attached to a kinesin motor travelling along a microtubule orientated in the Y-axis. c) 3D iso-energy profile of bead centre positions in the bound state highlighted on graph.

1924-Pos

Multiple Interacting Kinesin-1 Motors Cooperate Negatively

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Many sub-cellular commodities are transported by more than one motor, and it is well-known that the combined function of motors can lead to unique transport behaviors. Yet, little is known about how grouping multiple motor proteins influences the motile properties of cargos, and in particular, relationships between the structural / compositional organization of motor complexes and key collective transport parameters (run lengths, detachment forces) have not been established.We have taken important steps towards solving this problem by synthesizing the first set of structurally-defined complexes of interacting kinesin-1 motors. Furthermore, we have developed 'single-molecule' assays that can examine new and important aspects of collective motor dynamics; namely, whether multiple motors cooperate in a positive or negative fashion and if these behaviors influence ensemble transport properties of multiple motor systems. Herein, we demonstrate that interactions among two elastically-coupled kinesin molecules lead to negative motor cooperativity, and that this behavior influences collective motor force production. We also describe how such effects can reconcile differences between measurements of cargo motions *in vitro* and in living cells.

1925-Pos

Kinesin-1's Behavior at Obstacles

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Using single molecule stepping assays, we were able to show that kinesin-1 stops when it encounters an obstacle in its path on the microtubule lattice. Based on the stepping mechanism of kinesin-1, we propose the following model to explain why the molecule stops at obstacles:

Kinesin-1's processivity requires the rear head to stay bound until the leading head is firmly attached to the next tubulin dimer. The fact that kinesin-1 follows a single protofilament limits the choice of forward binding to the next tubulin dimer along the same protofilament. Therefore, if a large molecule is blocking the next tubulin dimer, the leading head cannot bind and the rear head cannot detach. This situation effectively stalls the kinesin-1 molecule until it detaches from the microtubule or a forward binding site becomes free.

Based on this model, we were able to calculate the dissociation rate of kinesin-1 in the stopped state. This calculated value agreed very well with a direct measurement, indicating that the model accurately describes kinesin-1's behavior at obstacles. A very similar dissociation rate has been measured previously for single-headed kinesin-1 mutants, suggesting that kinesin-1 waits at obstacles in a one-head bound state.

Interestingly, in about 50 % of the observed stopping events, kinesin-1 did not detach at the end of the stopping phase, but overcame the obstacle and continued to walk. The rate with which kinesin-1 exited the stopped phase by overcoming the obstacle was almost identical to the dissociation rate measured for stopping events. Therefore, it is likely that kinesin-1 overcomes a roadblock by detaching from the microtubule and then, instead of leaving into solution, reattaching next to, or behind the obstacle.

1926-Pos

***In Vitro* Analysis of the Effect of Microtubule Acetylation on Kinesin Motility**

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Plus end-directed intracellular transport by kinesins on microtubules in eukaryotic cells directs cargo to the cell's periphery, but to carry out polarized transport, additional signals from microtubules must be recognized by cargo-carrying kinesins. One emerging hypothesis, supported by *in vivo* observations of preferential kinesin-1 transport along acetylated microtubules, suggests that post-translational modifications (PTMs) of tubulin subunits in subsets of microtubules serve as markers for intracellular transport. Here we are examining if and how acetylation of microtubules directly regulates kinesin motility. As a source of acetylated and unacetylated microtubules, we have used *Tetrahymena* doublets extracted from a wild type strain and a mutant strain wherein the otherwise acetylated Lysine-40 is mutated to an Arginine. For obtaining fluorescently-labeled kinesin, lysates were extracted from COS cells transfected with Kinesin-1 genetically labeled with three-tandem monomeric citrines (3xmCit-KHC). To evaluate the effect of acetylation on Kinesin-1 motility, we used TIRF (Total Internal Reflection Fluorescence) microscopy to perform single molecule *in vitro* motility assays and measure the velocity and run length of 3xmCit-KHC on acetylated and unacetylated doublet microtubules. Our observations show that while the *in vitro* velocity remains unaltered, twice as many binding events can be observed for 3xmCit-KHC on wild-type doublets than on unacetylated doublets. We conclude that the motor domain of Kinesin-1 directly recognizes acetylation of microtubules and has a greater tendency to bind acetylated microtubules than unacetylated microtubules. We suggest that acetylation of microtubules enhances the binding affinity of Kinesin-1, which in turn allows preferential transport by Kinesin-1 along acetylated microtubules. To exclude differences between motility assays as source for the observed preferential binding of Kinesin-1 to acetylated microtubules, we are now comparing the binding and motility of Kinesin-1 for acetylated and unacetylated microtubules in the same motility assay.

1927-Pos

Surface Passivation for Molecular Motor Protein Assays

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In kinesin motiliy assays, it has been shown that the surfaces with which kinesin interacts must be passivated in order to prevent kinesin from denaturing on them. The most popular surface blocker is the casein family of milk proteins. Casein is usually purified to various degrees from bovine milk and has many unknowns associated with it when reconstituted and used in motor protein assays. In order to obtain a clearer picture of how kinesin and microtubules interact, a cleaner surface passivation needs to be found. The interaction of kinesin with microtubules has been studied extensively, however, there are fewer studies that investigate how the interaction of kinesin and microtubules changes due to surface passivation. One recent study has shown that the differing components of casein (termed alpha, beta, and kappa) can significantly affect microtubules in gliding motility assays [1]. Gliding motility assays are assays where a glass cover slip is passivated and kinesin is prevented from interacting directly with the substrate. Microtubules are then propelled by the motor activity of a bed of immobilized kinesin molecules. Lipid molecules are fatty acids that can be purified to a much greater extent than casein can. Also, lipid molecules exhibit the same amphiphilic behavior as casein, they adhere to glass easily, and can be easily functionalized. Lipids are thus an attractive alternative